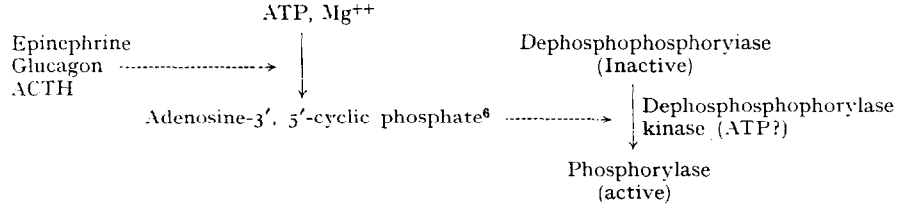
An enzymic defect in ascites-tumor cells*

Although glycogen phosphorylase is found in almost all mammalian tissues, greatly reduced phosphorylase activities recently have been ascribed to ascites tumors[1] and a solid tumor[2]. The following hormonally-dependent sequence of reactions been proposed for phosphorylase activation in liver and adrenal cortex[3-5].



This communication demonstrates a complete absence of phosphorylase activity in eight different types of tumors. The cells were found to contain phosphorylase-activating enzymes but lacked specifically both active and inactive forms of phosphorylase, and therefore, they did not respond physiologically to epinephrine or glucagon.

The following ascites tumors were used: Hepatoma, Ehrlich carcinoma, lymphocytic leukemia, plasma cell, mast cell, Krebs-2 carcinoma, and sarcoma-37; also the HeLa carcinoma grown in tissue culture. Freshly harvested tumors contained very low levels of glycogen, approximating 5 μmoles glucose equivalents/g protein, as determined analytically by the method of STADIE, HAUGAARD AND MARSH[7]. Histochemical examination with HIO_4-Schiff reagent indicated that the glycogen was confined entirely to normal polymorphonuclear leukocytes which comprised 1–2 % of the ascites-cell populations. Ascites-tumor cells grown *in vivo* and the HeLa carcinoma grown in tissue culture contained no detectable glycogen. In contrast, normal mouse-liver epithelial cells grown in tissue culture are known to contain glycogen[8].

The phosphorylase activities of whole, undialyzed tumor and normal mouse-liver homogenates were determined by the method of SUTHERLAND AND WOSILAIT[4]. An active phosphorylase was found in mouse-liver homogenates (1.53 μmoles phosphate released/mg protein/10 min). Phosphatase activity was negligible. Greatly decreased phosphorylase activities were found in hepatoma homogenates ($<$ 0.1 μmole phosphate/mg protein/10 min). Optimal activity was obtained between pH 6–7. Addition of 5′-AMP did *not* result in increased phosphorylase activity, nor did the use of a wide variety of homogenization techniques. Similar results were obtained with the previously listed tumors. A histochemical phosphorylase assay[9] was applied to the hepatoma, Ehrlich carcinoma and Krebs-2 carcinoma. An active phosphorylase was found in the polymorphonuclear leukocytes; none was present in the tumor cells. The phosphorylase reaction was then measured in the reverse direction, from glycogen to G-1-P, by following the disappearance of added glycogen

* The following abbreviations are used: 5′-AMP, adenylic acid; ATP, adenosine triphosphate; G-1-P, glucose-1-phosphate; G-6-P, glucose-6-phosphate; TCA, trichloroacetic acid; TRIS, tris (hydroxymethyl) aminomethane; DPP kinase, dephosphophosphorylase kinase.

from the medium. Normal mouse-liver and -muscle homogenates rapidly degraded glycogen; tumor homogenates did not degrade glycogen appreciably. The anaerobic utilization of G-1-P and G-6-P by tumor homogenates was compared manometrically. G-1-P was utilized as effectively as G-6-P, indicating phosphoglucomutase activity (20–30 μl CO_2/mg dry wt. tumor homogenate/h).

Addition of 50 μg epinephrine·HCl/ml medium to intact Ehrlich cells and 50 μg epinephrine·HCl + 25 μg glucagon/ml medium to hepatoma cells did not activate a phosphorylase. Addition of both hormones to mouse-liver slices resulted in a 20 to 60% reactivation of this enzyme.

Fig. 1 demonstrates that HeLa-carcinoma homogenates rapidly reactivated added dog dephosphophosphorylase. The activation process was proportional to the amount of homogenate added, was ATP dependent, and no activation occurred when a boiled homogenate was used. Addition of dog dephosphophosphorylase kinase did not greatly increase the rate of activation. Similar results were obtained with Ehrlich carcinoma, hepatoma and normal mouse-liver homogenates.

These data describe a unique type of glycogen storage disease, since the tumors possess the phosphorylase-activating enzymes, yet lack a functional dephosphophosphorylase. It is not known whether this defect occurs in non-neoplastic cells. All types of tumors tested resemble biochemical mutants insofar as all have a similar block in the normal hormonally-linked sequence of phosphorylase activation.

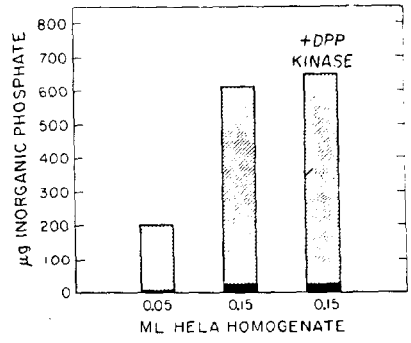


Fig. 1. Activation of dog dephosphophosphorylase by HeLa-Carcinoma homogenates. Cross-hatched bars refer to vessels containing dog dephosphophosphorylase. Solid bars represent identically prepared vessels with no dog dephosphophosphorylase. Reaction mixtures contained 4 μmoles TRIS, pH 7.4, 0.34 μmole ATP, 0.5 μmole $MgSO_4$, 0.039 μmole epinephrine·HCl, whole homogenate, and where indicated, dog-liver dephosphophosphorylase and dog-liver dephosphophosphorylase kinase. Total volume was 3.2 ml. Reaction mixtures were incubated at 30° for 5 min. 1 ml of the phosphorylase reagent[4] containing 2.0 μmoles 5'-AMP was then added and the tubes were incubated at 37° for 20 min. Samples were deproteinized by TCA precipitation at 0 and 20 min.

The help of Dr. SAMUEL SPICER for performing the histochemical essays and Dr. EARL SUTHERLAND for generously supplying purified preparations of dog-liver enzymes is very gratefully acknowledged.

Laboratory of Biochemistry and Metabolism, National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, Bethesda, Md. (U.S.A.)

MARSHALL W. NIRENBERG*

[1] M. W. NIRENBERG, *Federation Proc.*, 17 (1958) 283.
[2] A. A. HADJIOLOV AND K. I. DANCHEVA, *Nature*, 181 (1958) 547.
[3] T. W. RALL, E. W. SUTHERLAND AND J. BERTHET, *J. Biol. Chem.*, 224 (1957) 463.
[4] E. W. SUTHERLAND AND W. D. WOSILAIT, *J. Biol. Chem.*, 218 (1956) 459.
[5] R. C. HAYNES, JR. AND L. BERTHET, *J. Biol. Chem.*, 225 (1957) 115.
[6] D. LIPKIN, W. N. COOK AND R. MARKHAM, *Abstr. Am. Chem. Soc., San Francisco, Calif.* (1958) 4D.
[7] W. C. STADIE, N. HAUGAARD AND J. B. MARSH, *J. Biol. Chem.*, 188 (1951) 167.
[8] B. B. WESTFALL, V. J. EVANS, J. E. SHANNON, JR. AND W. R. EARLE, *J. Natl. Cancer Inst.*, 14 (1953) 655.
[9] T. TAKEUCHI AND H. KURIAKI, *J. Histochem. and Cytochem.*, 3 (1955) 153.

Received May 17th, 1958